

SEROPREVALENCE OF BRUCELLOSIS IN DONKEYS AND MULES IN PUNJAB, PAKISTAN

F. Wadood, M. Ahmad¹, F. Ahmad², D.H. Mughal³, M. Qamar⁴, Z.U. Hassan⁵, M.A. Gondal⁶, S. Hameed⁷, M. Ikram⁸ and S.G. Mohyuddin⁹

Department of Theriogenology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan; ¹Department of Theriogenology; University of Agriculture, Faisalabad, Pakistan; ²Livestock Production Research Institute, Bahadurnagar, Okara, Pakistan; ³Quality Enhancement Cell, University of Veterinary and Animal Sciences, Lahore, Pakistan; ⁴Department of Animal Nutrition, CUVAS, Bahawalpur, Pakistan; ⁵Department of Pathology, CUVAS, Bahawalpur, Pakistan; ⁶Institute of Continuing Education and Extension, CUVAS, Bahawalpur, Pakistan; ⁷Department of Anatomy and Histology, College of Veterinary Science, Islamia University, Bahawalpur, Pakistan; ⁸Directorate of Planning and Evaluation Livestock Department Government of Punjab, Pakistan, ⁹Department of Veterinary Medicine, Guangdong Ocean University, Zhanjiang 524088, China
Corresponding author's e-mail: wadood_114@hotmail.com

ABSTRACT: Donkeys and mules are neglected animals of livestock family. In donkeys and mules, brucellosis seroprevalence was checked in present study. A 22 mules and 160 donkeys serum samples were collected (Faisalabad and its surrounding area). After initial screening with Rose Bengal Plate test (RBPT), positive samples were again validated with Serum Agglutination test (SAT). A 4.4 and 3.75% brucellosis positive samples were recorded by RBPT and SAT, respectively in donkeys. However, this percentage was 9.1% by RBPT and SAT in mules. Source wise prevalence in donkeys was 0%, 33.3% and 3.2% from Faisalabad, Toba Tek Singh and private animals, respectively. In mules, prevalence of 11.8% (male) and 0% (female) whereas in donkeys, seroprevalence of 1.35% (male) and 3.4% (females) was recorded. Seroprevalence of 3.27% was recorded in donkeys of 1-5 years of age while 4.1% was noted in 6-10, 3.44% in 11-15 and 0% above 15 years of age. Prevalence of 8.82%, 3.84% and 2.08% was recorded in good, fair, and poor body conditioned donkeys, respectively. Whereas prevalence of 14.28 %, 12.5% and 14.28 % was noted in good, fair and poor body conditioned mules, respectively. Brucellosis prevalence was 8.83% in donkeys of foaling 0, 8.70% of foaling 1, 8.57% of foaling 2, 10% of foaling 3 and 12% of foaling above 3. In donkeys, all tested parameters (body condition, age, parity and sex) excluding source of samples have non-significant influence at brucellosis prevalence. All tested parameters were nonsignificant in mules.

Key words: Seroprevalence, brucellosis, equines, mules, donkeys.

(Received 12.01.2020

Accepted 10.03.2020)

INTRODUCTION

Brucellosis is caused by gram negative coccobacillus brucella bacteria and this disease results in huge financial losses (Radostits *et al.*, 2000) and mainly affects cattle, equines and humans.

After bacterial entry into body through gastric system, intact skin, lungs and mucous membranes causes local infection (Palunduz *et al.*, 2000) and disease pathogenesis depends on host immunity and bacterial multiplication potential (Gorvel, 2002; Fichi, 2003). Bacterial outer membrane has lipopolysaccharide that protect brucella from host immunity, endotoxicity, antimicrobial resistance and help in intracellular growth (Lapaque *et al.*, 2005).

B. canis (Aydin *et al.*, 1987), *B. suis* (Cook and Kingston, 1988) and *B. abortus* (Ocholi *et al.*, 2004) affects the equines with clinical signs of tenosynovitis, general infection, infertility, osteoarthritis (Denny, 1973),

poll evil, joint infection and fistulous withers (Izgur *et al.*, 1988).

In Pakistan, brucellosis control is not possible until disease status in all animals is not known (Ahmed and Munir, 1995a). There is no data available about the status of the disease in donkeys and mules in Punjab. Therefore, present study was planned to discover the status of brucellosis seroprevalence in donkeys and mules in Punjab, Pakistan.

MATERIALS AND METHODS

Animals: For present study, 22 mules and 160 donkeys belonging to military farms or private persons were chosen from Faisalabad and its surrounding area. Data of every animal about source, parity, sex, age and body condition was recorded. Sex wise, donkeys were distributed in male and female while regarding age donkeys were categorized into age < 15, 11-15, 6-10 and

1-5 years of age. Regarding body condition mules and donkeys were categorized in good, fair and poor whereas regarding parity, donkeys were divided into parity above 3, 3, 2, 1 and 0. Serum was obtained and kept at -20°C for future usage.

Analysis of Collected Serum Samples: Serum samples collected for disease diagnosis were initially tested with RBPT, already established procedure (Wadood *et al.*, 2009). Hyper-immune serum produced in rabbits was used to run positive and negative control trials for RBPT. Doubtful and positive serum samples via RBPT were again verified by SAT (Hussain, 2002), mentioned by (Wadood *et al.*, 2009). Antigen of both tests were purchased from VRI (Veterinary Research Institute), Lahore, Pakistan.

Analysis of Data: Chi-square was used for statistical analysis of data obtained.

RESULTS

By RBPT (4.4%) and SAT (3.75%) donkeys found positive for brucellosis whereas mules showed 9.1% disease prevalence by both tests. Disease seroprevalence was significantly different when all three sample sources were tested (Table 1). Disease prevalence by SAT in donkeys of Remount area Toba Tek Singh was 33.3%, 3.2% in private area and 0% in Remount area Faisalabad.

In donkeys, all other tested parameters (body condition, parity, age, and sex) have non-significant influence at brucellosis prevalence (Table 1). All tested parameters were nonsignificant in mules (Table 2).

Table 1. Seroprevalence of Brucellosis in Donkeys.

Factor	Description	No. of Samples	SAT positive Samples		Chi-square	P value
			No	%	Value	
Source	Remount Faisalabad	2	0	0	6.199	0.045
	Remount T.T. Singh	3	1	33.3		
	Private	155	5	3.2		
Sex	Male	74	1	1.35	0.745	0.388
	Female	86	3	3.4		
Age (Years)	1-5	61	2	3.27	0.253	0.969
	6-10	74	3	4.1		
	11-15	29	1	3.44		
	Above 15	5	0	0		
Body Condition	Poor	48	1	2.08	2.263	0.323
	Fair	78	3	3.84		
	Good	34	1	3.82		
	0	12	1	8.33		
Parity	1	23	2	8.70	0.382	0.984
	2	35	3	8.57		
	3	40	4	10		
	>3	50	6	12		

Table 2. Seroprevalence of brucellosis in mules.

Parameter	Description	Total No. of Samples	SAT positive cases		Chi-square	P value
			No	%	Value	
Sex	Male	17	2	11.8	0.647	0.421
	Female	5	0	0		
Body condition	Poor	7	1	14.28	0.014	0.993
	Fair	8	1	12.5		
	Good	7	1	14.28		

DISCUSSION

In present research, effort was done to discover seroprevalence of brucellosis in donkeys and mules, important but comparatively neglected livestock species.

Effect of body condition, sex, source of sample, parity and age on seroprevalence was noted. Contrary to present study, comparatively lower prevalence in mules (5.4%) was noted in another study (Safirullah *et al.*, 2014) that might be correlated to samples source. In donkeys, lesser

disease prevalence compared to another study (Safirullah *et al.*, 2014) might also be correlated to samples source. As out of 160 donkeys, a no of 155 were kept by private persons due to which animal's population size was small and wider choices of breeding females. Whereas all mules (22) were from army remounts farms with higher brucellosis prevalence. Similar results to present study have been reported (Bandara and Mahipala, 2002).

Regarding sex, similar results to present study in horses (Ardo *et al.*, 2016) and sheep, cattle and goat (Muma *et al.*, 2006) was noted. Different results to our study were recorded by (Safirullah *et al.*, 2014; Berbawy, 2012; Ahmed and Munir, 1995b; Njoga *et al.*, 2018) as in their studies, females showed significantly more brucellosis prevalence compared to males as during pregnancy and lactation females face more physiological stress and also remained in more contact to aborted fetuses and uterine discharges (Wadood *et al.*, 2009).

Regarding age, contrary results recorded several researchers (Berbawy, 2012; Ahmed and Munir, 1995b; Agab 1997; Kazi *et al.*, 2005); who recorded more disease prevalence in adults compared to young animals that may be correlated to the fact that brucella confine itself to the lymph nodes until erythritol is produced after conception and moreover young animals not show antibodies until parturition and abortion (Keppie *et al.*, 1965).

In relation to body condition, Comparable results were noted in another study (Ahmed and Munir, 1995b) in which no relation of body condition with brucellosis prevalence was recorded; but, in another study (Safirullah *et al.*, 2014) brucellosis prevalence in was significantly low in good body condition equines than poor body conditioned.

Brucellosis seroprevalence in relation to parity was non-significant and these results are similar to (Berhe *et al.*, 2007) in which brucellosis prevalence in relation to parity in cattle was non-significant.

Conclusion: In donkeys, all tested parameters (body condition, age, parity and sex) excluding source of samples have nonsignificant influence at brucellosis prevalence. Whereas, all tested parameters were also nonsignificant in mules.

REFERENCES

- Agab, H. (1997). Clinical signs of animal brucellosis in Eastern Sudan. *Rev Elev Med Vet Trop.* 50(2): 97-98.
- Ahmed, R. and M. A. Munir (1995a). Epidemiological investigations of brucellosis in horses, dogs, cats and poultry. *Pak. Vet. J.* 15(2): 85-88.
- Ahmed, R. and M. A. Munir (1995b). Epidemiological investigations of brucellosis in Pakistan. *Pak. Vet. J.* 15(4): 169-172.
- Ardo, M. B. D., M. Abubakar and Z. Adamu (2016). Prevalence of brucella antibodies in horses (*equus caballus*) in jalingo, taraba state, Nigeria. *J. Pub. Heal. Epid.* 8(7): 111-114.
- Aydin, N., J. Erdner and K. S. William (1987). Detection of *Brucella canis* antibodies in horses by the mercaptoethanol microagglutination test. *Vet. Microbiol. Der.* 6(1):95-100.
- Bandara, A. B. and M. B. Mahipala (2002). Incidence of brucellosis in Sri Lanka: An overview. *Vet. Microbiol.* 90(1-4): 197-207.
- Berbawy, S. M. (2012). Seroprevalence study on the current situation of brucellosis in horses and donkeys in assiut governorate. *Assiut. Vet. Med. J.* 58 (134): 323-327.
- Berhe, G., K. Belihu and Y. Asfaw (2007). Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray Region of Ethiopia. *Intern. J. Appl. Res. Vet. Med.* 5: 65-71.
- Cook, D. R. and G. C. Kingston (1988). Isolation of *Brucella suis* biotype 1 from a horse. *Aust. Vet. J.* 65(5): 162-165.
- Denny, H. R. (1973). a review of brucellosis in the horse. *Equine Vet. J.* 5: 121-125.
- Fichi, T. A. (2003). Intracellular survival of *Brucella*: defining the link with persistence. *Vet. Microbiol.* 92: 213-223.
- Gorvel, J. P. (2002). *Brucella* intracellular life: from invasion to intracellular replication. *Vet. Microbiol.* 90: 81-110.
- Hussain, M. (2002). A Laboratory Manual for Diagnosis of Animal Diseases. E. Team Publisher, Islamabad (Pakistan): 83-84p.
- Izgur, M., O. Akay, A. Candas, A. Inan, H. Ayhan and O. Esendel (1988). Study on the prevalence of equine brucellosis in Ankara. *Vet. Microbiol. Der.* 6: 117-126.
- Kazi, M., R. Amin, M. B. Rahman, M. S. Rahman, J. Han, J. Park and J. Chae (2005). Prevalence of *Brucella* antibodies in sera of cows in Bangladesh. *J. Vet. Sci.* 6: 223-226.
- Keppie, J., A. E. Williams, K. Witt and H. Smith (1965). The role of erythritol in the tissue localization of the brucella. *British J. Exp. Pathol.* 46: 104-108.
- Lapaque, N., I. Moriyon, E. Moreno and J. P. Gorvel (2005). *Brucella* lipopolysaccharide acts as virulence factor. *Curr. Opin. Microbiol.* 8: 60-66.
- Muma, J. B., K. L. Samui, V. M. Siamudaala, J. Oloya, G. Matope, M. K. Omer, M. Munyeme, C. Mubita and E. Skjerve (2006). Prevalence of antibodies to *Brucella* spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface

- areas of Zambia. *Trop. Anim. Hlth. Prod.* 38: 195-206.
- Njoga, EO., J. I. Onunkwo, S. O. Ekere, U. J. Njoga and W. N. Okoro (2018). Seroepidemiology of Equine Brucellosis and Role of Horse Carcass Processors in Spread of Brucella Infection in Enugu State, Nigeria. *Int. J. Cur. Res. Rev.* 10 (10): 39-45.
- Ocholi, R. A., J. K. P. Kwaga, I. Ajogi and J. O. O. Bale (2004). Phenotypic characterization of Brucella strains isolated from livestock in Nigeria. *Vet. Microbiol.* 103(1): 47-53.
- Palunduz, A., S. Palunduz, K. Guler and N. Guler (2000). Brucellosis in a mother and his young infant: probable transmission by breast milk. *Int. J. Infec. Dis.* 4: 55-56.
- Radostits, O. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff (2000). *Veterinary Medicine*, 9th Ed. W. B. Saunders Company Ltd; London (UK).
- Safirullah, K., A. Anwar, M. Raziq, S. Shahid, N. K. Raza and Y. Amin (2014). Epidemiological study of brucellosis in equines of district Peshawar Khyber Pakhtunkhwa Pakistan. *Int. J. Curr. Microbiol. App. Sci.* 3(2): 795-800.
- Wadood, F., M. Ahmad, A. Khan, S. T. Gul and N. Rehman (2009). Seroprevalence of brucellosis in horses in and around Faisalabad. *Pak. Vet. J.* 29 (4): 196-198.